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Association of Altered Brain Norepinephrine and Serotonin with the Obesity Induced by Goldthioglucose in Mice[1]

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COSCINA, D. V., R. A. McARTHUR, H. C. STANCER AND D. D. GODSE. *Association of altered brain norepinephrine and serotonin with the obesity induced by goldthioglucose in mice.* PHARMAC. BIOCHEM. BEHAV. 9(1) 123–128, 1978.—Two experiments examined the possibility that mice rendered obese by systemic injection of goldthioglucose (GTG) possess altered endogenous levels of brain norepinephrine (NE), dopamine (DA), serotonin (5-hydroxytryptamine or 5HT) and/or 5-hydroxyindoleacetic acid (5HIAA). In the first experiment, single-housed GTG-obese mice were found to have normal brain DA and 5HIAA but 14% less NE and 6% less 5HT than controls. This neurochemical profile was strikingly similar to that previously reported for rats rendered obese by ventromedial hypothalamic lesions (i.e., normal DA and 5HIAA, 19% less NE, 7% less 5HT). However, in the second experiment, equally obese GTG mice pair-housed with non-obese controls showed normal DA, 5HIAA, and NE but 9% more 5HT than controls. In other words, absolute levels of these brain substances were inconsistent with respect to obesity across experiments. On the other hand, when ratios of all possible combinations of these compounds were compared across experiments, only 5HT/NE ratios were consistently different (higher) in GTG mice. In addition, reliable inverse correlations were obtained between weight gain parameters and brain 5HT/NE or 5HIAA/NE ratios for GTG mice. These findings suggest that interactions between brain 5HT and NE neurons may contribute to the overeating and obesity which occur in mice after GTG administration.

Goldthioglucose Brain Dopamine Obesity Norepinephrine 5-Hydroxyindoleacetic acid Serotonin Transmitter interaction Mouse

BILATERAL lesions of the medial hypothalamus (MH) in or near the ventromedial nuclei are well known for their ability to elicit protracted overeating and obesity in a number of mammalian species. It has recently been shown that such lesions in rats also produce chronic decrements in endogenous concentrations of the putative brain neurotransmitters norepinephrine (NE) and serotonin (5-hydroxytryptamine or 5HT) [3, 4, 5, 8]. Of particular interest are observations that depletion of one or both brain monoamines (MAs) can inversely correlate with the magnitude of lesion-induced [3, 5, 8] or knifecut induced [9,18] weight gain. These findings, linked with abundant anatomical, biochemical and pharmacological data, implicate abberant brain NE and 5HT metabolism in the pathogenesis of over-nutritional obesity (for recent summaries see [1, 2, 11, 16].

Another frequently studied model of obesity is that induced in mice by systemic injections of goldthioglucose (GTG). The capacity of this compound to induce protracted hyperphagia and weight gain has been attributed to its relatively high affinity for and subsequent neurotoxic action on cellular elements within the MH axis (e.g. [15]). A number of behavioral similarities have been documented for MH-lesioned and GTG-obese rodents (e.g. [21,22]). Therefore, we wondered if GTG-treated mice would show abnormal brain NE and/or 5HT levels comparable to those seen in MH-lesioned rats. While some literature suggests that systemic GTG treatment can, in fact, alter brain NE and/or 5HT metabolism [7, 12, 13], the data were derived from several different species and are inconsistent as to the direction of neurochemical changes induced. Moreover, none of these studies were designed to examine the quantitative relationships which might exist between the magnitude of obesity

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and degree of brain amine change following GTG treatment. The present paper reports two experiments which specifically address this possibility.

GENERAL METHOD

Animals

A total of 112 adult, female Swiss-Webster (albino) mice purchased from Canadian Breeding Farms and Laboratory (Montreal, Quebec) were used.

Housing and Maintenance

For the duration of study, all mice were housed in rectangular (28 cm long, 18 cm wide, 13 cm deep) Isotab™ cages constructed of translucent plastic sides and bottoms and fitted with stainless-steel grid tops which also served as overhead feeders and water-bottle supports. The number of mice housed per cage varied for each experiment (see below). Bed-O'Cobs™ litter, which was used to cover cage floors, was changed weekly. Sufficient amounts of fresh Teklad pellet chow (4% fat) and tap water were always available to permit ad lib feeding and drinking except as noted below. All cages were kept in a temperature-controlled (23°C ± 2°) room illuminated 12 hr daily (lights on at 0800 hr) by a 100 W incandescent bulb recessed in the ceiling.

Injections

GTG (Schering Corp.) was administered in either oil or saline vehicles as described below. All GTG and vehicle control injections were administered intraperitoneally.

Preparation of Brain Tissue

At the end of all observation periods, mice were sacrificed by cervical dislocation. Whole brains caudal to the olfactory bulbs were dissected free from the calvaria, the pineal glands and meninges removed, and surface blood washed free in cool (4–10°C) saline. Brains were then weighed, wrapped in aluminum foil, frozen in liquid nitrogen, and stored in a freezer (–20°C) until assay. The time interval between sacrifice and freezing of brains was never longer than 3 min.

Brain Assays

Separate fluorometric assays for whole brain NE, 5HT, dopamine (DA) and 5HT's major metabolite, 5-hydroxyindoleacetic acid (5HIAA), were performed for each experiment. Brains and aqueous standards were homogenized in 10 volumes of cold acidified n-butanol, mechanically shaken for 5 min, centrifuged at 2000 rpm for 5 min, and the supernatant butanol extract retained. Ten ml of heptane + 0.8 ml of 0.1 N HCl were then shaken with 5 ml of this extract and centrifuged as before. The resultant organic phase plus 0.2 ml of the aqueous phase were retained for 5HIAA and 5HT determinations, respectively, as described by Maickel and co-workers [17]. A 0.4 ml aliquot of the aqueous phase was frozen until the next day when NE and DA were determined as described by Shellenberger and Gorden [23]. In this laboratory, recoveries of these compounds are high (at least 80%) and consistent (SEM=2–4% per assay) (see [4] for details of specific recoveries); therefore, values were not corrected for recovery.

Statistical Analyses

Data were analyzed with the aid of a PDP-8 computer (FOCAL programs) plus Texas Instruments Series 57 and 59 programmable calculators. All comparisons of group bodyweight (BW) and neurochemical differences were evaluated by *t*-tests for independent samples [10]. Appropriate correlational analyses were undertaken by calculating Pearson Product Moment Correlation Coefficients [10]. All p values reported represent two-tailed probabilities.

EXPERIMENT 1

The first experiment was designed to quantify the aforementioned brain amine profiles in single-housed GTG-treated mice allowed free access to food. These conditions were analogous to those used by us before for studying this same MA profile in obese MH-lesioned rats [3].

Method

Forty-eight mice (18–27 g range: $\bar{X}$ ± SEM=23.3 ± 0.63) were placed in individual cages as described above and allowed to eat and drink ad lib. After 4 days of such adaptation, 32 mice received 14 mg GTG in 0.7 ml vegetable oil (Unico™) while the remaining 16 received equal volumes of oil alone. Expressed as a function of BW, this drug dosage ranged from 0.52–0.78 mg/g. This dose was selected since it had previously been reported to induce histologically discernible MH damage and obesity [15]. Since such treatment produced substantial mortality within the first 3 postinjection days, 10 additional mice (15–24 g range: $\bar{X}$ ± SEM=21.2 ± 1.5 g, respectively) from the same shipment of animals were added to the study at this point. These latter animals were injected with a smaller dose of GTG (8 mg/mouse; range=0.33–0.53 mg/g) in 0.4 ml saline and deprived of food for 24 hr both prior to and after injection to reduce the drug's toxicity (see [14]). Prior to sacrifice, all mice were weighed two or three times weekly for a total of 31 (oil-injected) or 27 (saline-injected) days.

Results

Figure 1 shows the mean ± SEM BWs recorded for all three groups studied over the entire observation period. Regardless of GTG dose and/or vehicle by which it was administered, it is clear that surviving mice who received this drug gained more weight than controls. Statistical comparisons of GTG-oil vs. GTG-saline subgroups revealed no differences between starting BWs (23.3 ± 0.629 g vs. 21.2 ± 1.5 g, respectively) or terminal BWs (36.7 ± 1.52 g vs. 33.0 ± 1.61 g, respectively), hence their data were pooled. When all GTG mice were compared with vehicle-treated mice, it was found that while starting BWs did not differ (22.3 ± 0.798 g vs. 22.3 ± 0.555 g, respectively), terminal BWs for GTG mice were substantially higher (35.0 ± 1.18 g vs. 28.6 ± 0.797 g, respectively; $t(25)=4.5541$, $p<0.001$). This increment appeared even more pronounced after data were corrected for slight individual variations in starting weights and duration of study by expressing BWs as gs gained per day $\times 10^3$ (GTG mice=427 ± 39.9 vs. vehicle mice=204 ± 18; $t(25)=5.222$, $p<0.001$).

Comparison of brain assay results between GTG-oil vs. GTG-saline subgroups revealed no differences for any of the compounds measured. Consequently, these data were pooled and compared to brain assay results obtained from

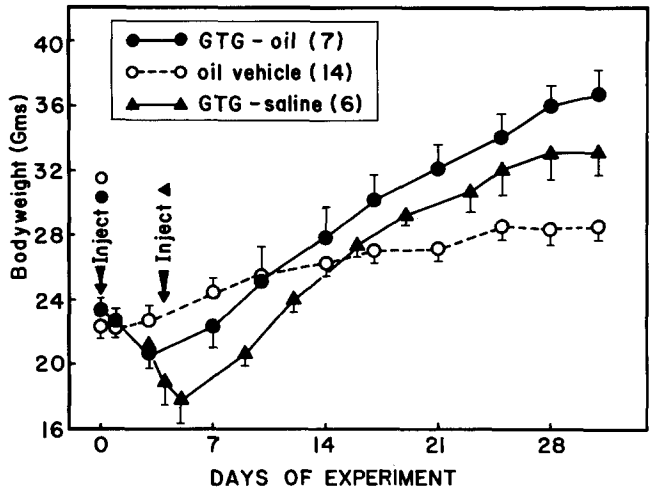

FIG. 1. Mean and standard errors of mean group bodyweights recorded in Experiment 1. See inset for definition of group symbols and number of animals per group. The days of the experiment at which intraperitoneal injections were administered are denoted by vertical (downward) arrows. The group receiving each particular type of injection is indicated by group symbol(s) immediately above the arrows.

TABLE 1

BRAIN ASSAY RESULTS FOR SINGLE-HOUSED GTG- VS. VEHICLE-INJECTED MICE

Group	N	NE	5HT	DA	5HIAA
GTG	13	385 ± 14*	969 ± 18	1236 ± 16	315 ± 10
Vehicle	14	445 ± 11	1029 ± 22	1217 ± 22	339 ± 10
	p	<.001	<0.06	NS	NS

* All values expressed as mean ± SEM ng/g concentrations

control mice. As shown in Table 1, brains from GTG-obese mice contained 13.5% less NE ($p<0.001$) and 6% less 5HT ($p<0.06$) than did brains from control mice. In contrast, no differences in DA or 5HIAA levels were observed. Correlation coefficients calculated between BW and individual brain chemical variables revealed no statistically significant findings.

Discussion

The results of this first experiment agree remarkably well with our previous neurochemical findings in single-housed ad-lib fed rats rendered obese by MH lesions [3]. In both experiments, obese animals possessed significantly less brain NE and 5HT but normal DA and 5HIAA as compared to control mice. Indeed, the magnitude and statistical reliability of depletions for both compounds compare favorably across MH lesion vs. GTG conditions (i.e., −19% NE and −7.4% 5HT, $ps<0.001$ and 0.01, respectively, in lesion study; −13.4% NE and −6% 5HT, $ps<0.001$ and 0.06, respectively, in present GTG study). However, unlike our previous MH lesion study, no statistically reliable correlations were found between the degree of BW gain and brain levels of endogenous NE and/or 5HT. This dissimilarity may simply reflect the relatively small sample size of obese animals here (N=13) as compared to earlier (N=40). Within this perspective, the similarities observed between experiments, despite rodent strain used or means of inducing obesity, support previous suggestions that these brain MA systems are importantly related to the development and/or maintenance of overeating (for discussion see [1, 2, 11, 16]).

EXPERIMENT 2

If the results of Experiment 1 are reliable, decreased brain NE and 5HT should be observed in additional mice rendered obese by GTG treatment. At the same time, such replication would permit expansion of the original sample size and reassessment of potential correlative relationships which might exist between BW and brain MA measures. With these considerations in mind, the second experiment was performed.

Method

Sixty-four mice (20–29 g range: $\bar{X}$ ± SEM=24.3 ± 0.28) of the same strain and sex as Experiment 1 were used. To accommodate this many mice in our laboratory at this particular time, it was necessary to house two mice per cage. After matching members of each pair for starting BWs and allowing one week acclimation to holding conditions, one mouse from each pair received 0.4 GTG/g BW in saline while the other member received the same volume of saline. As for GTG-saline mice in Experiment 1, all animals were deprived of food both 24 hr before and after injection to reduce GTG toxicity. All mice were weighed daily for the first 7 days postinjection, then once weekly for the next three weeks. Following this observation period, all surviving pairs of mice were sacrificed as before for determination of brain NE, DA, 5HT and 5HIAA.

Results

Of the 32 pairs of mice injected, only 16 pairs survived the full observation period. Figure 2 shows the mean ± SEM BWs recorded for both groups across the 28 day observation period. Again, it is clear that GTG treatment was effective in generating substantial weight gain. To statistically confirm this fact, it was determined that while starting BWs did not differ between GTG- vs. saline-injected groups (24.5 ± 0.516 g vs. 24.6 ± 0.444 g, respectively), terminal BWs for GTG mice were reliably higher (38.9 ± 1.11 g vs. 30.2 ± 0.540 g, respectively; $t(30)=7.0481$, $p<0.001$). As in Experiment 1, this difference was even more apparent by expressing individual BW data as gs gained per day × 10^3 (GTG mice=496 ± 29.2 vs. vehicle mice=190 ± 15.6; $t(30)=9.2431$, $p<0.001$). Of additional interest, comparisons of these latter BW data with those derived from mice in Experiment 1 revealed no reliable difference between the weight gains of GTG-treated ($t(27)=1.4267$) or vehicle treated ($t(28)<1$) groups.

The results of brain assay measures are summarized in Table 2. As in Experiment 1, no differences in DA or 5HIAA levels were observed. However, unlike Experiment 1, NE levels were unchanged from those of controls while 5HT levels were elevated 9.4% ($p<0.05$). Correlation coefficients calculated between BW gained and levels of each brain compound again failed to reveal significant results.

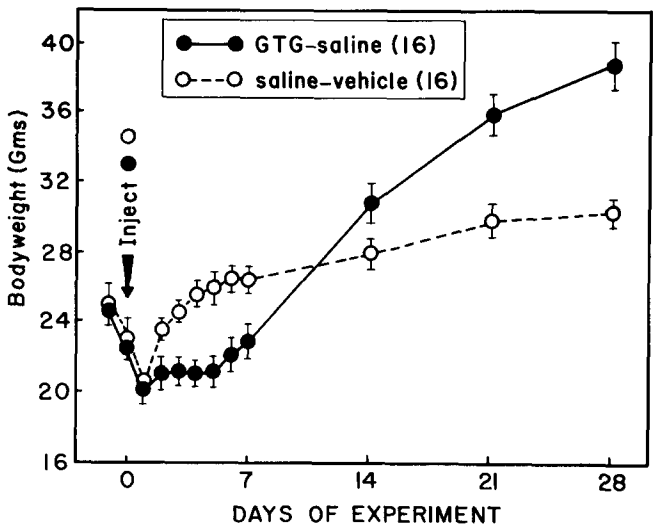

FIG. 2. Mean and standard errors of mean group bodyweights recorded in Experiment 2. The figure format is the same as described in Fig. 1 caption.

TABLE 2

BRAIN ASSAY RESULTS FOR PAIR-HOUSED GTG- VS. VEHICLE-INJECTED MICE

Group	N	NE	5HT	DA	5HIAA
GTG	16	529 ± 11*	896 ± 27	1347 ± 31	260 ± 6
Vehicle	16	524 ± 10	819 ± 24	1312 ± 28	258 ± 7
	p	NS	<0.05	NS	NS

* All values expressed as mean ± SEM ng/g concentrations

Discussion

Compared to Experiment 1, the most important finding in Experiment 2 was the quantitative divergence between GTG-induced obesity and brain MA profiles. That is, while GTG mice in both studies became equally obese and controls in both studies displayed the same (normal) BW accreation, endogenous levels of brain NE and 5HT differed relative to control values. More specifically, both brain NE and 5HT were depressed in Experiment 1, while brain NE was normal and brain 5HT was elevated in Experiment 2.

How can these differences be explained? Factors of GTG dosage per unit BW, vehicle for GTG administration and duration of food restriction do not appear responsible since they were equivalent for the subgroup of GTG-saline mice added to Experiment 1 and for all GTG mice in Experiment 2. The only difference between experiments was the housing density of animals, i.e., single-housed (isolated) in Experiment 1 vs. pair-housed (communal) in Experiment 2. These housing differences are potentially important since Welch and Welch [24] have reported that pair-housed mice possess higher rates of brain NE and 5HT turnover than do single-housed mice. However, accepting the possibility that such turnover differences could exist here between experiments does not account for our data. In the first place, Welch and Welch [24] also reported that brain DA turnover is accelerated by pair housing. We found no difference across studies in brain DA. Therefore, some unitary effect on these three brain amine systems previously shown responsive to housing [24] does not seem to be operating. Also, since control and GTG-treated mice for each study were housed under identical conditions, the observed GTG vs. control differences cannot be explained by interexperimental housing variations alone. Instead, some hitherto unknown interaction between GTG treatment and housing would have to be postulated. Even if such interaction did occur, it is unclear that differenial turnovers in GTG-treated mice would produce the altered levels of NE and 5HT measured here. The index of turnover used by Welch and Welch [24] was the accumulation of brain MAs following systemic administration of the MA oxidase inhibitor, pargyline. This index assumes that endogenous MA synthesis proceeds at whatever rate was present prior to degradative inhibition. However, no treatment used here would be expected to preferentially inhibit NE or 5HT breakdown. Therefore, the different source of altered MA levels between studies defies direct comparison. All these considerations make it unlikely that potential turnover changes attendant upon housing can uniformly explain our present neurochemical findings.

At face value, our finding of equivalent GTG-induced obesity being associated with different levels of endogenous brain NE and 5HT seems incompatible with substantial previous work implicating both neural systems in the control of feeding (for reviews see [1, 2, 11, 16]). However, such a restrictive interpretation is only merited if BW gain is predicted to co-vary with parameters of individual MA metabolites. Contrary to this view is accumulating evidence that these two neural systems can interact to modify brain chemistry and behavior (for example, see [20]). Therefore, we re-examined our neurochemical data with regard to relative rather than absolute amine differences.

Group data from both experiments suggested that 5HT levels were proportionately higher than NE levels in brains of GTG-obese mice relative to controls. To assess this statistically, all raw chemical data were transformed to percentages of mean control levels per experiment. From these scores, ratios of 5HT/NE $\times 10^3$ were computed for each animal. Since no group differences existed within treatments between experiments, data were combined for analysis. The mean ratio for all GTG mice (1102 ± 33.4, N=29) was 9.8% higher than the mean for all controls (1004 ± 19.2, N=30) which was statistically significant ($t(57)=2.565$, $p<0.02$). This finding supports the possibility that an altered interaction between brain 5HT and NE systems may have contributed to GTG-induced obesity.

To determine if some selective association appears to exist between the magnitude of GTG-obesity and this index of brain 5HT/NE interaction, correlation coefficients were calculated between BW gain data and all possible ratos derived from raw neurochemical data. The results of this analysis are shown in Table 3. Looking first at control (vehicle) animals, no reliable correlation was found between weight gain and any of the neurochemical ratios determined. For GTG mice, the magnitude of obesity correlated inversely with brain 5HT/NE ratios ($p<0.06$). The only other brain chemical ratio which reliably ($p<0.01$) correlated with BW increase was 5HIAA/NE. As with 5HT/NE ratios, the direction of this correlation was negative. Taken together with the group differences cited above, these findings provide tangible support for an interactive mode between brain 5HT and NE neurons in the genesis and/or maintenance of GTG-induced obesity.

TABLE 3

CORRELATION COEFFICIENTS BETWEEN G BW GAINED/DAY AND VARIOUS RATIOS DERIVED FROM BRAIN ASSAY RESULTS FOR GTG- VS. VEHICLE-INJECTED MICE FROM BOTH EXPERIMENTS

Group	N	5HT* 5HIAA	5HT NE	5HIAA NE	5HT DA	NE DA	5HIAA DA
GTG	29	+.1677	−.3560*	−.5128‡	−.1566	+.2447	−.2895
Vehicle	30	+.0569	−.0061	−.0557	−.1945	−.2176	−.1747

† All ratios calculated from percent mean control levels.
* $p<0.06$
‡ $p<0.01$

GENERAL DISCUSSION

Previous research has provided nominal evidence that GTG neurotoxicity affects brain 5HT and/or NE neurons [7, 12, 13]. Of particular interest is one fluorescence histochemical study of various NE- and 5HT-containing brain regions in GTG-treated rabbits [13]. A total of seven structures were studied: (1) nucleus supraopticus (hypothalamus), (2) nucleus periventricularis (hypothalamus), (3) median eminence, (4) raphe (midbrain), (5) substantia nigra, (6) locus coeruleus, and (7) cerebral blood vessels. During the first postinjection week, the NE and 5HT fluorescence in these regions tended to increase. This corresponded to a time of weight loss in rabbits much as was seen in GTG-treated mice observed here. Over the next three weeks, these rabbits displayed normal weight gain. This was associated with decreased regional NE and 5HT fluorescence. At this time, particularly marked fluorescence decrements were observed in the raphe and locus coeruleus—well-known sites of origin for 5HT and NE cell bodies, respectively, which send axonal projections to the forebrain. Unfortunately, the ordinal nature of these histochemical findings linked with an absence of GTG-induced obesity in rabbits limited conclusions that could be drawn from this work. Nevertheless, the implication that both types of MA neurons are putative targets for GTG action is compatible with our findings.

The primary stimulus for the present work was previous documentation of reliable 5HT and NE depletions in brains of MH-lesioned rats [3, 4, 5] which could correlate inversely with the magnitude of their obesity [3]. We reasoned that if similar findings were obtained in GTG-treated mice, the importance of either or both MA systems in feeding must be sufficiently robust to transcend species and independent variable differences. To that end, the present results are somewhat equivocal. On the one hand, the quantitative findings of Experiment 1 are in fairly good agreement with our earlier MH-lesion work (see Discussion of Experiment 1). The absence of similar quantitative findings in Experiment 2 can be ascribed to differential housing conditions as compared to both Experiment 1 and this lesion work. On the other hand, if we accept the additional suggestion of an inverse relationship between obesity and brain 5HT/NE ratios irrespective of housing, an inconsistency is seen between obesity models. More specifically, when we calculate from our previous lesion study [3] the correlation between gs BW gained per day and ratios of percent control brain 5HT/NE as done here, we obtain reliable positive correlation ($r(38)= +.3233$, $p<0.05$) rather than negative (see Table 3). Clearly, additional work is required to clarify these differences. In this respect, our observation of high correlation between BW gain and 5HIAA/NE ratios suggests that a more dynamic index of 5HT to NE metabolism may be helpful in understanding the central effects of GTG leading to obesity. If this speculation is correct, more meaningful interactions between MA systems can be studied by measuring the major metabolites of both systems. To so characterize brain NE requires quantifying levels of 3-methoxy-4-hydroxyphenylglycol (MHPG). This cannot be accomplished with the type of spectrophotofluorometric method used here. One further refinement in extensions of this work should include biochemical determinations confined to discrete brain regions. This latter suggestion is supported by (a) Ishizaki's [13] delineation of distinct areas along the various MA neuroaxes which appear differentially responsive to GTG treatment, and (b) documentation of additional extra-hypothalamic loci which appear reactive to GTG's neurotoxicity [6,19]. To accomplish such work in small brain samples (i.e., 10–50 mg tissue weight) requires extremely sensitive (i.e., 100–500 picogram) analytic capacity. Radioenzymatic methods can meet such criteria but are not well suited for metabolite determinations. The current method of choice involves gas-chromatography with mass fragmentographic detection. This means of analysis is currently being developed in our laboratories.

Aside from the theoretical considerations stimulated by our data with respect to central nervous system control over feeding and BW regulation, one practical point should be made. The neurochemical results obtained here make it clear that dissociation can occur between parameters of BW and endogenous MA levels. To the extent that these latter measurements are taken as specific indicators of brain neuronal mechanisms modifying ingestion, our data caution against making functional interpretations of such MA changes when only one general biochemical index is employed. At the same time, our data also suggest that comparisons of experimental findings irrespective of environmental variables such as housing density may lead to erroneous conclusions as to potential interrelations between biochemical and behavioral states.

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